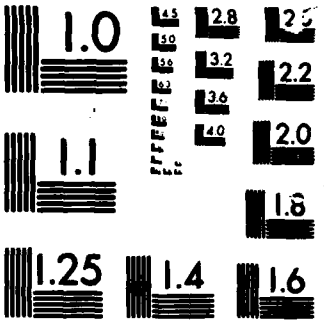
AD-A167 329 THE ROLE OF THE CYTOSKELETON IN INTESTINAL ABSORPTIVE FFNCTION AND RECEPTOR EXPRESSION IN THE CELLS(U) GEORGE WASHINGTON UNIV WASHINGTON DC M M CASSIDY 10 MAY 86 1/1

UUCLASSIFIED N00014-79-C-0603 F/G 6/16 NL



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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION (U)	1b RESTRICTIVE MARKINGS NA
2a SECURITY CLASSIFICATION AUTHORITY NA	3 DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited
2b DECLASSIFICATION/DOWNGRADING SCHEDULE NA	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) The George Washington University	5 MONITORING ORGANIZATION REPORT NUMBER(S) NA
6a NAME OF PERFORMING ORGANIZATION The George Washington University	6b OFFICE SYMBOL (If applicable) NA
7a NAME OF MONITORING ORGANIZATION Office of Naval Research	
6c ADDRESS (City, State, and ZIP Code) 2300 Eye St. N.W. Ross Hall Washington, D.C. 20037	7b ADDRESS (City, State, and ZIP Code) 800 N. Quincy St. Arlington, Va 22217-5000
8a NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research	8b OFFICE SYMBOL (If applicable) ONR
9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-19- C - 0603	
8c ADDRESS (City, State, and ZIP Code) 800 N. Quincy St. Arlington, Va 22217-5000	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 61153N; PROJECT NO: RR04108; TASK NO: NR 207; WORK UNIT ACCESSION NO:

11 TITLE (Include Security Classification)
The role of the cytoskeleton in intestinal absorptive function and receptor expression in the cells.

12 PERSONAL AUTHOR(S)
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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Final	FROM 7/1/79 TO 4/30/84	May 10, 1986	4 + 8 reprints

16 SUPPLEMENTARY NOTATION
Prepared by Dr. Marie M. Cassidy - N00014-79-C-0603 BM12640

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
08		

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Intestinal enterocytes/salts, solute, water absorption, Mucin secretion/gytoskeleton/microfilaments/ microtubules/ membrand receptors

19 ABSTRACT (Continue on reverse if necessary and identify by block number)
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20 DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	(U)
22a NAME OF RESPONSIBLE INDIVIDUAL Dr. J. A. Majde	22b TELEPHONE (Include Area Code) 202/696-4055
	22c OFFICE SYMBOL ONR

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE

Report on DOD/Navy/ONR Contract

N00014-79-C-0603

(BM 12640)

The role of the cytoskeleton in intestinal absorptive function and receptor expression in the cells.

The objective of this series of investigations was to explore and define the role of the cytoskeleton apparatus in the function and transport of solutes across the intestinal epithelium. Most epithelial cells, including the intestinal enterocyte and the mucin-secreting goblet cell are now known to possess a highly ordered cytoskeletal matrix comprised of microfilaments, intermediate filaments and microtubules. Macromolecular aggregates such as actin and a variety of related proteins are present in the amplification structures, i.e. microvilli of the mucosal surface. This component of the system was perturbed by the application of Cytochalasin B (CB), a macrolide antibiotic which interacts with mechanochemical proteins of microfilaments. Studies from a number of laboratories suggest that these cytokinins inhibit the rate of actin filament polymerisation and disrupt actin-based networks in a very dynamic manner. Work performed on this contract provides evidence that microfilaments and/or microtubules may be critical physicochemical transducers in the sequence of events leading to (1) transmural absorption of salts, organic solutes and osmotically-linked fluid flow; (2) mucin secretions by the goblet cells, which constitute approximately 20-30% of the surface cell population; (3) the expression of β receptors in HeLa cells by butyrate treatment is preceded by a dramatic reorganization of the intracellular cytoskeleton.

1. Serosal CB ($1\text{-}5 \times 10^{-5}$M) evoked a significant rise in electrical resistance and a 25% decrease in hydrogen ion secretion by frog gastric mucosa. Resistive effects but not acid secretion modification was readily reversible. In vivo absorption of sodium, glucose and water by rat jejunum was inhibited by 50% during steady state transport periods during which luminal CB was present. The results can be suitably explained by an electrical model wherein the microfilament-disruptive action of CB is represented by changes in a cytoplasmic resistance. In a second study, similar alterations in electrical potential difference, resistance and H^+ secretion rate were clearly a reflection of the structural state of the cell layer. A theoretical paper presented at a conference of investigators supported by ONR Biophysics Program at Virginia Polytechnic Institute in 1980 concluded that the range of absorption rates of isosmotic fluid across epithelia represents the need for energy-dependent

transfer of fluid volume units as opposed to solute units per se. It may be appropriate to reconsider earlier models of a mechanical volume pump for transcellular relocation of fluid volume units, encompassing a cytoplasmic component. This model should permit a flexible specificity with respect to the actively transported solutes and obviously incorporate the presence or transport of Na^+, Cl^-, HCO_3^- ions. Morphological evidence utilizing scanning and transmission electron microscopy provides directional polarization and a structured pathway for the secretory transport system in the gastric parietal cell and the absorptive transport mechanism in the intestinal cell. Cytochalasin-treated epithelia revealed a significant widening of the junctional regions between contiguous cells, which are normally tightly opposed. Filamentous thread-like material was present on the apical cell surfaces and between cells and was of such morphometric dimensions as to permit the preliminary conclusion that it represented disarrayed or disgorged filaments.

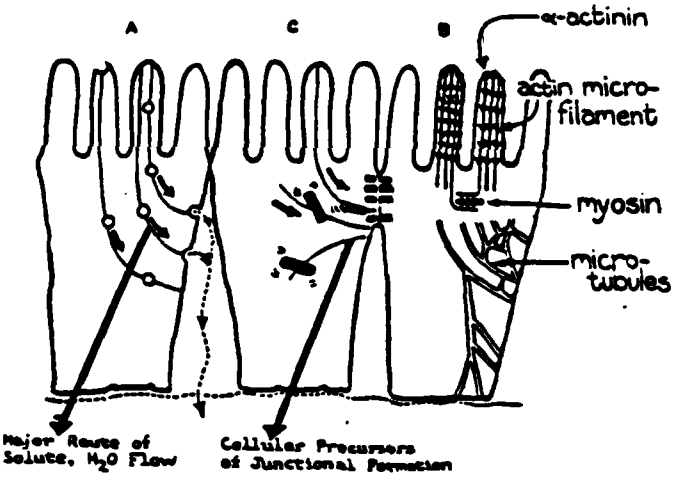

Fig. . This schematic diagram of intestinal epithelia illustrates 3 major concepts: A shows the major pathway for fluid absorption which is dependent on actively transported solutes. B represents our current understanding of the cytoskeletal scaffold present in these cells. It has not yet been demonstrated that the microfilaments present in the microvilli are architecturally contiguous with the intermediate filaments and the microtubules. C indicates that junctional formation and hence paracellular permeation characteristics may involve regulation by the cell in terms of microfilament assembly and transposition to the junctional region between individual cells.

The foregoing figure summarizes a possible model (B) which incorporates our experimental findings. Other investigators have demonstrated the strong role of the parietal cell cytoskeleton in the tubulo-vesicular movement of acidic volume units to the secretory apical surface of the stomach. Hence, we conclude that the spectrum of effects observed in these two transporting epithelia is compatible with the concept of involvement of mechanochemical element(s) in the active secretion of $[H^+]$ by frog gastric mucosa and isotonic transport of solutes and fluid by the in vivo rat intestine.

2. Structural-functional considerations with respect to epithelial transport mechanisms in organs exposed to external environments have rarely included a significant population of cells. Mucin synthesizing and secreting cells include most of the surface layer in the gastric and colonic loci and a fifth to a third of the mucosal barrier in the small intestine. The maintenance of a surface coat of mucus and its role in cell cyotprotection against acid or other noxious agents is now recognized to be a continuous rapid and dynamic mechanism. In an extensive investigative series aimed at defining the ultrastructural features of gastrointestinal mucosa subjected to cytoskeletal disruption, aspirin, prostaglandin or bile acid challenge morphometric findings clearly pointed to modifications of normal mucus secretory patterns.

These observations were followed by biochemical studies of mucus elaboration, in vivo, in a rat model. In almost all cases, the structural and biochemical methodologies yielded corroborative data. The ultrastructural techniques deployed were light microscopy, transmission and scanning electron microscopy. With these techniques in addition to blunt cryofracture of tissue specimens, correlative information concerning the detailed microarchitecture of the mucosal epithelial layers was derived.

Mucus-producing cells are clearly identifiable sub-populations of cells in many of the epithelial transport model systems studied; i.e. gastric, jejunal, and colonic epithelial of the rat. The distribution of these cells varies from a predominant location on the mucosal surface of the stomach to a regular degree of interspersion among the dominant columnar epithelial cell population in the small and large intestine. The functional state of these cells can be assessed by a variety of morphological techniques and their biochemical synthetic and secretory capacity estimated by isotopic tracer procedures. In the gastric or jejunal acute ulceration model, sulfo-

mucin production is decreased by aspirin and markedly enhanced by Prostaglandin E_1, providing additional evidence for the cytoprotective role of the mucin blanket. With chronic feeding of the bile salt sequestrants which provoke colonic mucosal irritation and injury, there is also stimulated mucin output. Disruption of the cytoskeletal framework of the mucosal cells by Cytochalasin B or colchicine depresses mucin production and secretion. The implication of these studies is that the microfilament/microtubular sub-structure of the mucosal cell plays a role in the functional vectorial conveyance of materials across the mucosal layer whether the moieties involved be absorbed electrolytes and water or a secreted organic component, e.g. mucin.

3. In a final phase of this project, the role of the cellular microtubular array in the induction of β-adrenergic receptors of the cell membrane was probed in the HeLa cell line. Preliminary attempts to visualize the cytoskeleton in isolated enterocytes from the small intestine proved frustrating because of altered morphological integrity. Butyrate is a potent inducer of functional alterations in many cultured cells. These include induction of β-adrenergic and cholera Toxin receptors in HeLa cells, hormone and enzyme synthesis, metabolic modifications and shape changes. Using a HeLa cell line (ES-1), we report a three-fold functional induction of β-adrenergic receptors by 5 mM sodium butyrate and a five-fold increment in l-isoproterenol-stimulated cAMP production following 24 hours of butyrate treatment. By indirect immunofluorescence microscopy using polyclonal and monoclonal antibodies untreated HeLa monolayers were observed to possess an extensive network of randomly arranged microtubules (MTN). In the presence of 5 mM butyrate for 24 hours, the previously reported elongate shape change was reflected in a reorganization of the MT system such that a majority were then oriented in the long axis of the cell, extending to the tips of the neurite-like processes or between them and few were seen in the vicinity of the nucleus. Essentially similar results were obtained with cells grown in chemically defined, serum-free medium. Colchicine (10^{-7}M) prevented the butyrate-induced shape and MT alterations. With colchicine alone a diffuse staining pattern, due perhaps to the presence of depolymerized tubulin, was evident. Cycloheximide (10 µg/ml) was without effect per se but blocked the butyrate-induced alterations in shape and MT patterns. It is concluded that the induction of β-adrenergic receptors and shape alterations in HeLa cells by butyrate is associated with a dramatic reorientation of the cytoskeleton, which is perhaps related to membrane insertion of the known pool of intracellular receptors. Modification of protein synthesis and subsequent modification of cell function may then ensue.

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